Original

Microwave-Irradiation of Lignocellulosic Materials X†

Conversion of Microwave-Irradiated Agricultural Wastes into Ethanol

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(Received August 31, 1989)

Abstract——Agricultural wastes, including rice straw, rice hulls and sugar cane bagasse, pretreated by a continuous microwave-irradiation were converted into ethanol. Almost all hemicelluloses were autohydrolyzed during the pretreatment at 210–220°C. Addition of acetic acid (0.5% concentration) into irradiation medium permitted lowering temperature by 10 to 30°C. Most of cellulose in pretreated samples of rice straw and bagasse was more effectively digested by cellulases because of the increase in their enzymatic susceptibility. Removal of hemicelluloses had, however, no direct connection with the increase of digestibility. Glucose produced by enzymatic saccharification were completely converted into ethanol. Yields of ethanol produced per kilogram (dry weight) of the pretreated samples of rice straw and bagasse were 378 ml and 285 ml, respectively.

Since the sever conditions of pretreatment to increase the enzymatic susceptibility reqiured the considerable amount of electric power, the milder condition to reduce the energy consumption for ethanol production was determined in this report.

Key words: continuous microwave-irradiation, removal of hemicelluloses, enzymatic digestibility, ethanol production, energy consumption

1. Introduction

Not only wood lignocellulose but also agricultural wastes, including rice straw, rice hulls and sugar cane bagasse, have been persistently receiving keep attention as raw materials for production of ethanol. The microwave-irradiation pretreatment[1] has been investigated for processing the lignocellulosic materials. It causes depolymerization of lignin and autohydrolysis of hemicellulosic polysaccharides so effectively to enhance enzymatic susceptibility. Azuma et al.[2] and Ooshima et al.[3] reported that the microwave-pretreatment was effective for conversion of agricultural wastes to the substrates for hydrolyzing enzymes. In generally, electricity is transformed into microwave with 50–60% efficiency. A severer conditions,

† Report IX: Mokuzai Gakkaishi, **34**(5), 462–468 (1988).
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which brought about rather decrease in production of pretreated substrates, were not shown to be ideal on account of lower energy efficiency.

In this investigation, using agricultural wastes as raw materials, we examined the energy consumption observed in pretreatment, and determined the most appropriate condition for the reduction of total energy consumed for ethanol production.

2. Materials and Methods

2.1 Lignocellulosic materials

Rice straw, rice hulls and sugar cane bagasse used for this experiment, were ground with separately to pass though a 5.6 mesh sieve, and then extracted with an ethanol-benzene mixture (1:2 v/v).

2.2 Microwave-irradiation pretreatment

Ground sample (air dried, 1 kg) was poured into a container with 15 l of water. After soaking for 3 hours, the water-sample mixture was fed to the microwave-irradiation apparatus at different flow rates (8–20 l/hr) to be irradiated continuously. The microwave used for irradiation had a frequency of 2,450 MHz and 4.9 kW maximum-output. Heating temperatures (160–225°C) were controlled by changing the output power of microwave generator and the flow rate of mixture[4].

The irradiated samples were washed thoroughly with distilled water to remove autohydrolyzates, with care taken to prevent them from drying. The residual matters thus obtained were used as the substrates for further saccharification and fermentation.

2.3 Measurement of monosaccharide composition of pretreated samples

Monosaccharide composition of the pretreated samples was determined after hydrolysis with 3% - H_2SO_4 according to Japan TAPPI No. 42–84.

2.4 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with cellulase preparation (Meicelase, Meiji Seika CEPB5042) in essentially the same way as reported one[5].

The enzyme (200 mg) was added to a reaction mixture with 2 g of pretreated sample (dry weight basis) and 100 ml of 0.05 M acetate buffer (pH 4.8). The reaction mixture was incubated with agitation for 48 hr at 40°C in the presence of a few drop of toluene. After the reaction time, the enzyme reaction was stopped by heating at 120°C for 15 min. Reducing sugars resulted from the enzymatic hydrolysis were determined by use of the 3,5-dinitrosalycylic acid method[6].

2.5 Alcohol fermentation

Alcohol fermentation[4] was carried out as follows. Nutritional salts were added to the sugar solution produced by enzymatic hydrolysis and the pH was adjusted to 5.5. To this solution (20 ml), yeast suspension (2 ml) was added. The fermentative substrate mixture was incubated with slight agitation at 30°C for 24 hr.

After the fermentation, ethanol in the substrate mixture was determined by a high-performance liquid chromatography (HPLC) on a column of AMINEX HPX-87H (Bio Rad Lab.).

3. Results and Discussion

3.1 Removal of hemicelluloses by pretreatment

Wong et al.[7] reported that autohydrolytic pretreatment brought about hemicelluloses-removal from lignocellulosic raw materials and resulting increase of enzymatic susceptibility. Previously, we reported that hemicelluloses of woody samples

Table 1. Conditions of continuous microwave-irradiation pretreatment and neutral monosaccharide composition of pretreated residue.

Sample	Final temp. (°C)	Flow rate (l/hr)	Pressure[a] (kgf/cm^2)	Output[b] power (%)	Weight[c] loss (%)		Monosaccharide composition[d] In water			In 0.5% AcOH solution		
							Ara	Xyl	Glc	Ara	Xyl	Glc
Rice straw	Un[e]	—	0.0	0	—[f]	—[g]	4.3	27.3	68.4	—	—	—
	180	14.8	11.5	62	10.1	(15.0)	4.3	26.1	69.6	3.2	20.5	76.3
	190	14.4	14.0	62	12.1	(16.0)	4.1	25.1	70.8	2.1	17.6	80.3
	200	14.4	17.0	100	13.8	(23.8)	3.2	20.5	76.3	0.6	7.3	92.1
	210	14.0	20.5	100	17.5	(27.0)	2.1	17.6	80.3	0.5	5.4	94.2
	220	10.8	24.5	100	24.0	—	0.5	7.4	92.1	—	—	—
	225	9.6	27.0	100	27.5	—	0.6	5.2	94.2	—	—	—
Rice hulls	Un	—	0.0	0	—	—	2.0	22.0	76.0	—	—	—
	180	13.8	11.5	62	5.0	(10.2)	1.5	20.8	77.0	1.0	18.1	80.9
	190	13.2	14.0	62	6.9	(15.3)	1.4	20.3	78.3	0.8	14.9	84.3
	200	12.3	17.0	100	11.9	(20.7)	0.5	15.3	83.7	0.4	7.1	92.6
	210	10.2	20.5	100	19.8	(22.1)	0.0	10.7	89.3	0.0	4.0	96.0
	220	9.8	24.5	100	22.5	—	0.0	4.3	95.7	—	—	—
	225	8.1	27.0	100	24.0	—	0.0	2.3	97.7	—	—	—
Sugar cane bagasse	Un	—	0.0	0	—	—	1.2	31.6	67.2	—	—	—
	180	12.6	11.5	62	8.6	(15.3)	1.2	30.4	68.4	1.0	11.4	87.6
	190	12.0	14.0	62	10.8	(16.5)	0.0	30.5	69.5	0.5	7.0	92.5
	200	12.0	17.0	100	15.5	(24.3)	0.0	27.7	72.3	0.3	4.4	95.3
	210	11.4	20.5	100	21.3	(26.5)	0.0	24.5	75.5	0.3	3.9	95.8
	220	11.2	24.5	100	26.1	—	0.0	10.2	89.8	—	—	—
	225	10.0	27.0	100	28.2	—	0.0	5.2	94.8	—	—	—

a) Processing pressure of pretreatment. b) The microwave-genetator has 4.9 kW/hr of output power at 100%. c) Based on the original sample weight. d) Relative amount of monosaccharides in pretreated residue. e) Untreated sample for control. f) Pretreated in water. g) Pretreated in 0.5% AcOH solution.

were effectively removed by the pretreatment of microwave-irradiation at 210°C[4]. In this investigation, the same pretreatment system was also applied to the agricultural waste samples.

Table 1 shows the conditions of continuous microwave-pretreatment and the sugar composition of pretreated samples. Weight loss, which indicates water soluble fraction containing mainly autohydrolyzed hemicelluloses, progressively increased with increasing temperature. The relative amount of glucose (%) in rice straw and bagasse reached 92.1% and 89.9% at 220°C, because of the removal of hemicelluloses, whereas the corresponding untreated samples (control) yielded glucose of 68.4% and 67.2%, respectively. Rice hulls released hemicelluloses at 210°C and yielded 89.3% glucose, whereas the untreated sample gave 76.0%.

Since the processing pressure of pretreatment required 24.5 kgf/cm^2 at 220°C as shown in Table 1, a considerable amount of electric energy was consumed for microwave-irradiation, and this is a large disadvantage for the pretreatment process. Therefore, attempts to reduce the processing temperature and pressure were made by addition of acetic acid (0.5% concentration) to the water-sample mixture. The successful results for removal of hemicelluloses are given in Table 1. The relative amount of glucose in rice straw yielded 92.1% at 200°C, which was compared to the degree achieved at 220°C in water. The decrease in heating temperature from 220°C to 200°C resulted in the reduction of 7.5 kgf/cm^2 of processing pressure accompanied by the increase in flow rate by 3.6 l/hr. Similarly addition of acetic acid to raw samples of rice hulls and sugar cane begasse permitted to drop the hemicelluloses-removal temperatures by 10°C and 30°C, respectively.

3.2 Enzymatic saccharification of pretreated waste samples

The extents of saccharification are expressed as yields of reducing sugars formed by enzymatic hydrolysis and based on the total carbohydrate of pretreated samples. As shown in Fig. 1, the extents of saccharification of both rice straw and bagasse increased with increasing in temperature, amounting to 98.8% and 98.1% at 220°C, respectively. The extent of saccharification of rice hulls pretreated at 225°C was 49.3%, which was obviously lower than those of rice straw and bagasse. The limited-increase in the extent of saccharification in rice hulls is probably due to presence of large amount of lignin and silicate strengthening cell wall[8].

When acetic acid was added to the water-sample mixture, the extents of saccharification of samples increased. However, comparing at the temperatures maximizing the hemicelluloses-removal, the extents of saccharification of rice straw and bagasse decreased from 98.8% to 60.1% and 98.1% to 63.2%, respectively. We assume that the pores produced by the removal of hemicelluloses are too small to accept the enzyme molecules[9], and other factors, including condensation reaction of liginin,

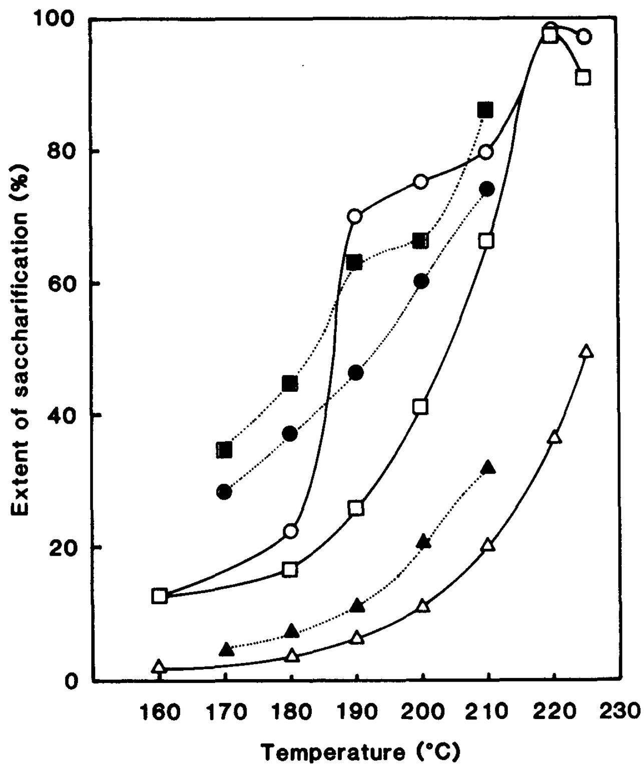

Fig. 1. Microwave irradiation of agricultural wastes in the presence of water or 0.5% AcOH solution.
Note: Extent of saccharification are based on the total carbohydrate of pretreated residue.
Symbols: (○, ●) rice straw, (△, ▲) rice hulls and (□, ■) sugar cane bagasse. Open symbols: the samples pretreated in water, Closed symbols: the samples pretreated in 0.5% AcOH solution.

rather affect the increase of enzymatic susceptibility[10]. Therefore, the increase in the extents of saccharification do not correspond directly with the temperature of hemicelluloses-removal.

3.3 Alcohol fermentation

Table 2 shows the yields of ethanol formed by simple fermentation. The yields which were independent of the temperature of pretreatment were 70–80%, 55–60% and 60–65% for rice straw, rice hulls and sugar cane bagasse, respectively. Since part of the sugars supplied are used for cell growth of yeast, the actual yield is about 87–90% at the maximum[11]. Therefore, almost all sugar from rice straw was consumed in the production of ethanol.

When the pretreatment was carried out in the 0.5% acetic acid solution, the low yields of ethanol were observed at the temperatures between 160°C and 180°C. In the range of high temperatures between 200°C and 225°C, however, the yields

Table 2. Yield of ethanol from microwave-pretreated samples by ethanol fermentation.

Sample	Final temp. (°C)	Initial glucose content[a] (%)	Yield of ethanol[b] (%)
Rice straw	180	75.5[c] (78.2)[d]	66.8[c] (34.6)[d]
	190	78.6 (85.3)	74.3 (71.0)
	200	80.8 (92.9)	71.4 (76.2)
	210	82.7 (96.8)	72.7 (78.0)
	220	93.6 —	80.8 —
	225	95.3 —	75.7 —
Rice hulls	180	75.2 (79.0)	26.7 (74.7)
	190	72.8 (80.1)	55.9 (70.1)
	200	80.5 (90.2)	63.4 (74.7)
	210	81.6 (93.9)	61.4 (69.8)
	220	89.2 —	54.2 —
	225	90.5 —	61.8 —
Sugar cane bagasse	180	69.2 (85.5)	55.4 (28.2)
	190	71.1 (90.4)	59.4 (31.4)
	200	74.6 (95.7)	64.1 (36.1)
	210	75.6 (96.3)	64.9 (40.6)
	220	88.7 —	58.7 —
	225	87.3 —	67.9 —

a) Determined by Glucose-Test Wako (WAKO PURE CHEMICAL Ind., LTD.) and based on the reducing sugars formed by enzymatic hydrolysis. b) Based on the theoretical amount from the initial glucose content in this table. Since part of glucose are used for cell growth of yeast, the actual yield is about 87–90% at the maximum. c) Pretreated in water. d) Pretreated in 0.5% AcOH solution.

increased to 71-78%, 70-75% and 31-41% for rice straw, rice hulls and bagasse, respectively. In comparision with the pretreatment in water, the conspicuous decrease in ethanol production was found only in the cause of bagasse sample. The decrease may be improved by the investigation of the conditions for fermentation.

3.4 Evaluation of ethanol production

To evaluate the productivity of ethanol, the production of ethanol from 1 kg of pretreated sample (dry weight basis) was calculated in Table 3.

For rice straw, the process involving the stages of substrate preparation and fermentation allows the output of 378 ml ethanol from the sample pretreated at 220°C in water. When the pretreatment is done in 0.5% acetic acid solution at 210°C, producible ethanol slightly decreases to 330 ml.

For rice hulls, the combination of enzymatic hydrolysis and fermentation allows

Table 3. Ethanol production from one kilogram of microwave-pretreated samples.

Sample	Temp. (°C)	Output of ethanol (ml)	Yieldof ethanol[a] (%)
Rice straw	190	242[b] (150)[c]	61[b] (35)[c]
	200	236 (243)	57 (46)
	210	249 (333)	60 (59)
	220	378 —	81 —
	225	353 —	75 —
Rice hulls	190	10 (27)	3 (8)
	200	23 (57)	7 (15)
	210	42 (81)	11 (22)
	220	65 —	18 —
	225	103 —	28 —
Sugar cane bagasse	190	56 (94)	18 (19)
	200	102 (121)	29 (24)
	210	166 (179)	49 (35)
	220	265 —	57 —
	225	285 —	57 —

a) Based on the theoretical amount from glucose content of pretreated samples. b) Pretreated in water. c) Pretreated in 0.5% AcOH solution.

the output of 103 ml ethanol from the sample pretreated at 225°C in water. Addition of acetic acid to the water-sample mixture decrease the outputs of ethanol to 81 ml. Therefore rice hulls are not suitable substrate for ethanol fermentation owing to much less productivity than that of rice straw.

For sugar cane bagasse, the sample pretreated in water is converted into 285 ml ethanol by the combination of enzymatic hydrohysis and fermentation. Addition of acetic acid in the water-sample mixtuue gives rise to the reduction of producible ethanol to 179 ml.

Table 4 shows the producible ethanol from waste substrates pretreated for one hour. Since the condition of the fermentation and enzymatic hydrolysis is fixed, the productivity of ethanol is affected directly by the flow rate of pretreatment. For instance, the pretreated rice straw (10.8 l/hr) at 220°C allows 174 ml ethanol production. However, also at 190°C, the sample (14.4 l/hr) can produce 172 ml of ethanol. A difference of 30°C produces the difference of only 2 ml ethanol.

As shown in Table 1, the output power of microwave generator required 62% to maintain the temperature at 190°C, but keeping the temparature at 220°C required the saturation level. The data indicate that the ethanol production per output power become 2.8 ml at 190°C and 1.7 ml at 220°C. For the purpose of

Table 4. Producible ethanol from samples pretreated for one hour and per output power.

Sample	Temp. (°C)	Producible ethanol (ml) For one hour[a]	Per output power[b]
Rice straw	190	172[c] (102)[d]	2.8[c] (1.6)[d]
	200	164 (150)	1.6 (1.5)
	210	161 (190)	1.6 (1.9)
	220	174 —	1.7 —
	225	138 —	1.4 —
Rice hulls	190	7 (17)	0.1 (0.3)
	200	14 (31)	0.1 (0.3)
	210	19 (36)	0.2 (0.4)
	220	28 —	0.3 —
	225	36 —	0.4 —
Sugar cane bagasses	190	33 (47)	0.5 (0.8)
	200	54 (57)	0.5 (0.6)
	210	81 (78)	0.8 (0.8)
	220	119 —	1.2 —
	225	107 —	1.1 —

a) Calculated ethanol was based on glucose in samples pretreated for one hour. b) Producible ethanol from the samples pretreated for one hour was divided by output power in Table 1. c) Pretreated in water. d) Pretreated in 0.5% AcOH solution.

ethanol production, pretreatment at 190°C is appropriate.

Rice hulls show very poor productivity of ethanol. Calculated 0.4 ml of ethanol per output makes the cost factor of process more unrealistic. Segar cane bagasse shows 1.2 ml ethanol per output, whereas the productivity seems to be insufficient compared with that of rice straw.

In summary, although the microwave-irratiation pretreatment permits lignocellulose to enhance the enzymatic susceptibility, the pretreatment requires large amount of input electricity. Increase in the flow rate of sample under mild condition, however, will mitigate the energy consumption of microwave-irradiation.

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